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14. ABSTRACT

This proposal aims to test the hypothesis that integrating observations derived from mouse model systems with observations from human prostate cancers will define relevant and consistent molecular alterations critical to the development and progression of prostate carcinoma. The research accomplished to date has: 1) assembled the requisite mouse models to enable the generation of tumor gene expression data; 2) produced a second-generation mouse prostate microarray that will allow for deeper profiling of mouse prostate gene expression; 3) identified a specific gene (osteopontin) commonly associated with multiple mouse prostate cancer models; 4) developed the methods/techniques that will enable precise dissection of mouse prostate epithelium; 5) expanded the Prostate Expression Database to archive microarray data; 6) determined strain-specific gene expression differences in the mouse prostate that could contribute to phenotypic differences on prostate cancer development and progression; and 7) identified developmental pathways altered in the Pten-/- prostate cancer model that could contribute to the process of carcinogenesis.

15. SUBJECT TERMS
Database, mouse model, gene expression, microarray, transcript, proteomics

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Table of Contents

INTRODUCTION

This proposal aims to test the hypothesis that integrating observations derived from mouse model systems with observations from human prostate cancers will define relevant and consistent molecular alterations critical to the development and progression of prostate carcinoma. Ultimately, these studies will identify those mouse models most accurately reflecting *in vivo* human prostate cancer, and prioritize those genes in human prostate cancer that are most relevant for therapeutic intervention.

The aims of the proposal are unchanged. They are: (1) To determine transcript expression profiles of neoplastic lesions from mouse models of prostate carcinoma. (2) To stratify mouse models of prostate carcinoma through comparative analyses with clinical human prostate carcinomas. (3) To extend the utility of the Prostate Expression Database to facilitate comparative studies of mouse and human prostate carcinoma. (4) Write final report. (Note the original Aim 2 involving proteomic studies was deleted due to recommendations by the reviewers).

Disease relevance: Model systems represent critical resources supporting essentially all facets of research involving prostate cancer including studies focused on disease etiology, disease progression, diagnostics, dietary factors, immune modulation, imaging, and pharmacologic intervention. Mouse models offer opportunities for testing hypotheses that would be difficult or impossible to evaluate in humans. Similarly, databases of sequence, gene expression, and disease model information also greatly facilitate scientific work in an extremely cost- and time-effective manner. There is a crucial need to develop interactive resources that generate, compile, and distribute relevant data correlating mouse prostate cancer models directly with phenotypes and genotypes of human prostate carcinoma so as to interpret experimental findings in the appropriate context, determine disease relevance, and prioritize model systems for appropriate pre-clinical studies. This proposal aims to address these needs.

BODY

The following summarizes the technical objectives for the proposal and the work accomplished during the 24-month interval between the start of the project (12/06/04) and the preparation of this report (12/18/06).

D.1.Technical objective 1: To determine transcript expression profiles of neoplastic lesions from mouse models of prostate carcinogenesis (Months 1-24).

Objective 1a. Microdissect specific epithelial populations of cells at discrete stages of prostate carcinogenesis: PIN, invasive carcinoma, metastasis.

Task 1: Breed and microdissect PIN models (months 1-12). We have obtained and bred mouse prostate cancer models of the following genotypes: Nkx3.1-/-, and acquired prostates with PIN lesions from the PB-RXR-/- mouse. The FGF8 mouse is no longer available (Dr. Roy-Burman, personal communication). Thus we will use the expression profile from the Akt-/- mouse developed by Dr. William Sellers as an alternative. We have acquired gene expression data from prostates of these mice which develop PIN but not invasive cancer. We have now microdissected PIN lesions from the Nkx3.1-/- prostates. Objective completed.

Task 2: Breed and microdissect PIN and progression models (months 12-24. We have acquired, bred, and harvested prostates at the PIN and invasive cancer stages from the PB-PTEN-/- and TRAMP models. Microdissection of these lesions is complete as well as comparable wild-type controls. Objective completed for primary tumors. Metastatic tumors have been harvested and are awaiting microdissection.

Objective 1b. Measure transcript levels in specific epithelial populations of cells at discrete stages of prostate carcinogenesis: PIN, invasive carcinoma, metastasis.

*Task 3: Construct microarrays (months 1-6).*We have constructed a 2nd generation mouse prostate microarray that now comprises ~16,000 cDNAs representing ~12,000 unique genes. This array has been quality checked for sequence accuracy and reproducibility. Preliminary experiments using microdissected cancerous mouse prostate epithelium demonstrated high quality hybridization results. Task completed.

Task 4: Amplify RNA from microdissected mouse prostate tissue (months 6-24). We have microdissected normal and neoplastic mouse prostate epithelium from the PB-PTEN and TRAMP models, amplified the RNA, verified the quality of the aRNA using the Agilent bioanalyzer. Task completed.

Task 5: Hybridize mouse prostate cDNA probes to microarrays (months 6-24). We have hybridized amplified RNA from cancerous and benign mouse prostate epithelium in a comparative manner. The preliminary analysis of the PTEN-/- and TRAMP experiments identified ~600 genes differentially expressed between benign and neoplastic cells, with both lobe-specific and tumor-type specific differences (Fig 1). Analyses of these data are in progress as is verification of relevant gene expression differences using qRT-PCR and IHC.

We have finalized a study evaluating strain-specific differences on mouse prostate gene expression that may identify host differences accounting for different cancer penetrance rates. The manuscript was submitted to *Genome Biology* and is now in review. We have also identified pathways of normal mouse prostate development that are re-activated in the setting of neoplasia. We are now confirming the altered expression of these genes by qRT-PCR. The mechanistic analysis of one gene we found to be altered in multiple prostate cancer models, osteopontin, was published in *Cancer Research*.

Objective 1c. Identify expression alterations that are common to specific stages of neoplastic growth.

Task 6: Format and QC microarray data (months 18-24). Data acquired. QC complete. Very good quality and reproducibility across biological and technical replicates. Task complete.

Task 7: Statistical analyses of microarray data (months 18-24). Data acquired. Analysis in progress.

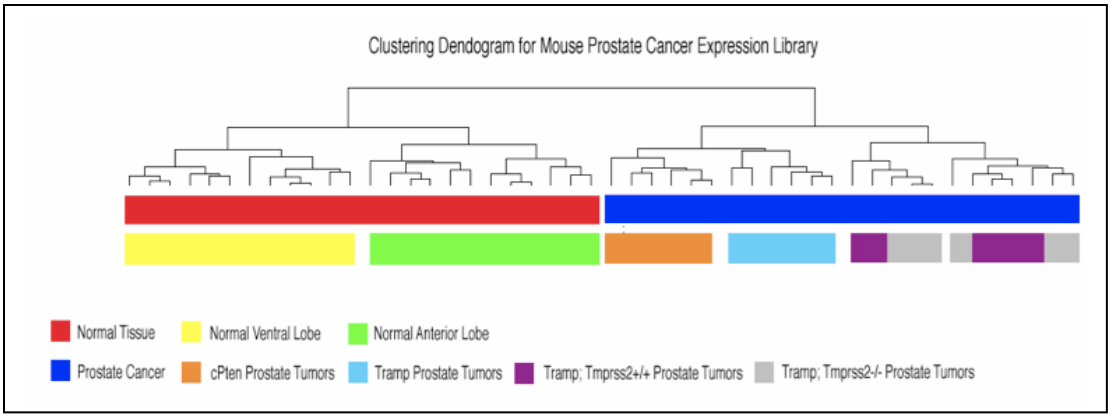

Figure 1. Dendrogram of gene expression similarities across microdissected epithelium of different genotypes (benign, Pten-/-, TRAMP) and anatomic location (prostate lobes). The clustering of samples is highly reproducible both with biological and technical replicates and clearly distinguishes the different anatomic and genetic components of the epithelial cells.

D.3. Technical objective 2: Stratify mouse models of prostate carcinoma through comparative analyses with clinical human prostate carcinomas (months 18-34).

Objective 2a *Determine transcript alterations that statistically-associate with specific models of mouse prostate neoplasia (e.g. $NKX^{-/-}$ vs $Pten^{-/-}$)*

Task 8: Analyze mouse model transcript profiles using SAM and ANOVA to identify gene expression changes associating with specific genotypes and histology (months 18-30). The data has been acquired for the Nkx, Pten, and TRAMP models at all stages. Our preliminary statistical analyses have identified >600 transcript alterations. Confirmatory studies are in progress.

Objective 2b Categorize human prostate tumors into classes based upon expression profile similarities to mouse models (e.g. $Pten^{\downarrow}$-like vs $Myc^{\uparrow}$-like);

Task 9: Acquire human prostate cancer microarray profiles (in-house and from data repositories on the www), and format for analyses (months 30-32). We have acquired human microarray datasets from 4 published studies: LaPointe et al, Singh et al, Stephenson et al and Yu et al. Task complete.

Task 10. Normalize mouse prostate cancer model expression microarray data with human normal prostate and cancer expression arrays (months 30-33). The datasets have been normalized and orthologs across array platforms and species have been mapped. Task complete.

Task 11: Compare mouse prostate cancer expression microarray data with human normal prostate and cancer expression arrays using the methods of Golub *et al* for class predictors (months 30-34). In progress.

Task 12: Create a list of alterations in prostate carcinoma that can be used to stratify prostate cancers in a clinical setting (months 32-35). In progress.

Objective 2c *Categorize human prostate tumors into classes based upon the expression levels of specific gene(s)* (e.g. over expression of *Myc*);

Task 13: Repeat tasks 11-12 using specific gene expression levels as discriminators (months 30-34). Pending completion of Task 11-12.

D.4. Technical objective 3: To extend the utility of the Prostate Expression Database (PEDB) for comparative gene expression studies of mouse and human prostate carcinoma. (months 10-34)

Objective 3a. Construct an interactive repository for microarray information that integrates multi-dimensional data (tissue type, quantitative and temporal gene expression measurements) for independent analyses.

Task 14: Determine a server configuration for a microarray database server (month 10). We have selected a server configuration using the opensource Bioconductor platform written in the 'R' language. We have begun populating the database with microarray data generated from the mouse model experiments described above.

Task 15: Install and update server configuration for security and accessiblity (month 10). Task Completed.

Task 16: Reconfigure the PEDB website to use PHP for faster data access and improved interactivity. (months 10-18). Task Completed.

Task 17: Evaluate "Minimum Information about a Microarray Experiment" (MIAME) compliant database/management systems: (months 12-18). Task Complete. All submitted mouse microarray data is now MIAME compliant.

Task 18: Implement database structure changes to make database suitable for microarray raw data, ratio data, proteomics, and image storage. (months 12-18). With the exception of the proteomics data, the database structure now houses microarray datasets.

Task 19: Organize and enter microarray data into chosen database and create file structure system for easy retrieval, analysis and visualization. (month 16-22). In progress.

Task 20: Identify mouse and human orthologs using the homologene database to determine matched pairs of genes. (months 14-22). Merge files for mouse microarray data and 2 different human array platforms: spotted cDNA and Affymetrix, have been completed that link orthologous genes. Task Complete.

Task 21: Create a Graphical User Interface for viewing and navigating between microarray overview, graphs, clustering programs, and sequence information. (months 18-26). In Progress.

Task 22: Write scripts to automate the input of new microarrays, including a web-based entry system, and creation of new graphs on a monthly basis. (months 20-28). In Progress.

Objective 4: Final Report: Complete data analyses, compile accomplishments and reportable outcomes and write final project report (Months 35-36).
This objective is planned for completion in months 35-56.

KEY RESEARCH ACCOMPLISHMENTS

- Completed the construction and q/c of a second generation mouse prostate specific microarray that nearly doubles the gene expression representation relative to version 1.
- Acquired the mouse prostate cancer models with specific genetic alterations leading to PIN or invasive cancer (Nkx3.1, RXRalpha, PTEN-/-, TRAMP) and gene expression data from the mouse prostate Akt model.
- Completed the wet-lab experiments evaluating strain-specific differences in mouse prostate gene expression that could influence the development and/or progression of genetically-engineered prostate cancer. A manuscript describing these results has been submitted to *Genome Biology* and is under review.
- Completed microdissection, amplification, and microarray analysis of benign epithelium, PIN and invasive carcinoma from the anterior and ventral lobes of the PTEN-/- and TRAMP mouse prostate cancer model systems. This analysis has identified several developmental pathways that appear to be re-activated in prostate adenocarcinoma (e.g. Wnt and Notch pathways) and determined profiles that associate with the specific initiating events in the course of cancer progression.

REPORTABLE OUTCOMES

Ani C. Khodavirdi, Zhigang Song, Shangxin Yang, Hong Wu, Colin Pritchard, Peter Nelson, and Pradip Roy-Burman. (2006). *Increased Expression of Osteopontin Contributes to the Progression of Prostate Cancer.* *Cancer Research*. 66(2):883-8.

Colin Pritchard, Madhuchhanda Bhattacharjee, Sarah Hawley, Ruth Dumpit, Robert Sikes, and Peter S. Nelson. *Gene Expression Patterns of Androgen-Regulated Prostate Development: Implications for Prostate Carcinogenesis* (*submitted: Genome Biology*).

Brig Mecham, Cynthia Heinlein, Roger Coleman, Michael Risk, and Peter S. Nelson. Gene expression correlates of mouse prostate carcinogenesis: alternative pathways corresponding to initiating events. (*In Preparation*).

CONCLUSIONS

The research accomplished to date has: 1) assembled the requisite mouse models to enable the generation of tumor gene expression data; 2) produced a second-generation mouse prostate microarray that will allow for deeper profiling of mouse prostate gene expression; 3) identified a specific gene (osteopontin) commonly associated with multiple mouse prostate cancer models; 4) developed the methods/techniques that have enabled precise dissection of mouse prostate epithelium; 5) expanded the Prostate Expression Database to archive microarray data; 6) determined strain-specific gene expression differences in the mouse prostate that could contribute to phenotypic differences on prostate cancer development and progression; 7) identified developmental pathways altered in the Pten-/- and TRAMP prostate cancer models that could contribute to the process of carcinogenesis; 8) Identified specific alterations corresponding to different tumor initiating events (e.g. Pten vs p53) which may have the potential to stratify human prostate cancers according to genetic lesions (in progress).

REFERENCES

None

APPENDICES

None.